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REPORTS

Mammalian Stratum Corneum Contains Physiologic Lipid Thermal Transitions

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Using a new high-sensitivity differential scanning calorimeter, capable of very slow scanning rates and large sample volumes, we examined the thermal transitions in neonatal mouse stratum corneum. Both physiological and supraphysiological transitions were found in intact tissue that were displaced on cooling and obliterated by solvent treatment establishing them as lipids. Physiologic peaks were encountered in lipid extracts from the same tissues. With heating and cooling recycling we found a novel effect of thermal "fractionation" of the peaks into discrete subfractions that appeared to correspond roughly the the number of bands found on thin-layer chromatography of the lipid extracts.

In recent years there have been several published descriptions of the thermal transitions in mammalian stratum corneum [1–4]. In general, these have revealed only supraphysiologic (above 70°C) transitions that have been attributed primarily to proteins [3–5]. None of these studies observed thermal transitions in the physiologic range, and, moreover, even when peaks were ascribed to lipids [3–5], no cooling data were presented. All these DSC experiments were done at extremely rapid scan rates (20°C/min) and low sensitivity (5 mcal/sec full-scale sensitivity). Furthermore, none of these earlier studies attempted to correlate presumed lipid transitions with thermal analysis of lipid extracts from the same tissue.

In this study, we have applied a new high sensitivity differential scanning calorimeter, at slow scan rates, to freshly obtained sheets of neonatal mouse stratum corneum. We describe herein lipid phase-transitions in both the physiologic and supraphysiologic ranges. Furthermore, in studying lipid extracts from the same tissue, we found that repeated heating and cooling recycling of the sample resulted in fractionation of the physiologic peak into several discrete bands.

MATERIALS AND METHODS

Preparation of Stratum Corneum Sheets

For animal studies, neonatal mouse skin was utilized because it is hairless and therefore essentially free of pilosebaceous lipids, and because sheets comprising viable corneum can be obtained from living neonatal mouse without chemical treatment by prior injection of the staphylococcal epidermolytic toxin [6]. The stratum granulosum is then removed, without perturbation of the stratum corneum, by incubating these sheets granular-layer-downward over filter paper impregnated with 0.5% trypsin in phosphate buffered saline for 2 hr at 37°C. The granular cells are then easily removed by gentle vortexing in PBS. To assess the impact of the trypsinization step on the data presented here, samples of stratum corneum, with stratum granulosum cells still adherent, were prepared and run in parallel to the homogeneous sheets—the results were virtually identical for both types of samples.

Preparation of Solvent Extracts and Thin-layer Chromatography (TLC)

Total lipids were extracted by the Bligh-Dyer method [7] and collected as described previously [8]. The whole lipids were subfractionated and recovered quantitatively after sequential separation of: (a) phospholipids in chloroform:methanol:water:glacial acetic acid (60:35:4.5:0.5, vol); (b) neutral lipids in petroleum ether:diethyl ether:glacial acetic acid (80:20:1, vol); and (c) glycosphingolipids in chloroform:methanol:water (90:10:1, vol) followed by petroleum ether:diethyl ether:glacial acetic acid (70:50:1, vol). Bands were visualized by spraying with 0.5% 8-anilino-1-naphthalene sulfonic acid (ANS) and photographed prior to weighing.

High Sensitivity Differential Scanning Calorimeter

The high sensitivity differential scanning calorimeter (DSC) used in this study was a Hart Scientific Model 7701-9001 DSC. Calibration of the DSC using highly purified n-alkanes (nC_{18} through nC_{36}) as a function of scan rate is reported in reference 9. A scan rate of 0.1°C/min at a full scale sensitivity of 0.4 m cal/sec was used in all experiments described in this communication. A 1.0 cm cell was used and approximately 30 mg of sample. The whole sheet stratum corneum was hydrated to 0.54 mg H_20/mg SC. All samples were run under a blanket of argon.

RESULTS

Examination of the DSC scan of whole, hydrated (54% water) stratum corneum sheets from neonatal mice revealed the peaks displayed in Fig 1. A broad transition is seen in the physiologic range, with a maximum at 38.2°C, and two higher thermal transitions between 60–70°C. In the cooling cycle these peaks are displaced downward suggesting supercooling of lipids (Fig 1). Assignment of these peaks as lipids is further supported by the obliteration of these peaks by solvent-extraction (data not shown).

When the total lipid extract from the same sheets (also hydrated, but containing no free water) was scanned up to 90°C, 2 major peaks were observed (37.7°C, 43.9°C) on the first heating scan (Fig 2). On the subsequent cooling scan, we found 5 major, sharp thermal transitions (Fig 3). As shown in Fig 2, when the lipid extract was rescanned (heating), 5 sharp transitions and 2 broad transitions (29°C, 44°C) appear. Recooling for a second time, however, did not result in any further change in the thermogram.

To rule out degradation or lipid modifications during the process of heating and cooling, we compared the thin-layer choromatographic profile of the original lipid extract vs. the extracts following the calorimetry procedure described. No new bands were observed on the TLC plate after heat recycling (Fig 4). It is also of interest to note that the number of fractions on

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Abbreviations:
ANS: 8-anilino-l-naphthalene sulfonic acid
DSC: differential scanning calorimeter
PBS: phosphate buffered saline
TLC: thin-layer chromatography

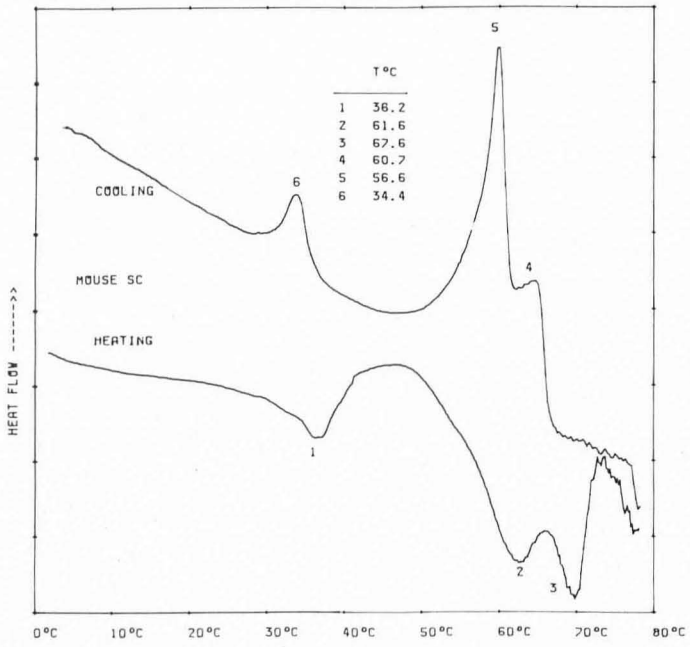

FIG 1. DSC heating and cooling scans of a typical neonatal mouse stratum corneum (SC) sheet. Scan rate 0.1°C/min and a full scale sensitivity of 0.4 mcal/sec.

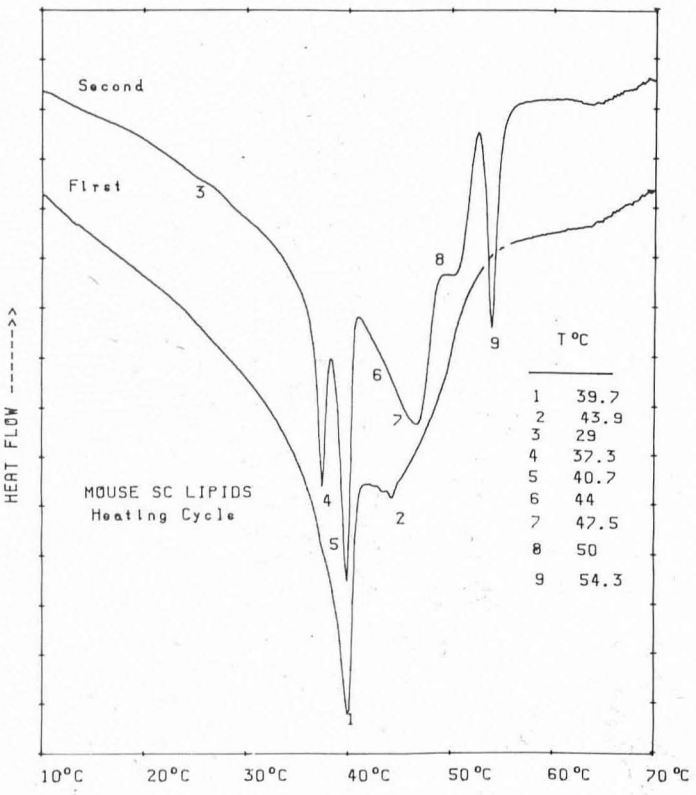

FIG 2. Recycling DSC heating scans of the lipid extract of neonatal mouse stratum corneum at the same scanning rate used for SC sheets. The cooling cycle is shown in Fig 3.

TLC approximates the number of peaks appearing on cooling and reheating in the calorimeter.

DISCUSSION

This study demonstrates several phase-transitions in both physiologic and supraphysiologic temperature ranges in neonatal mouse stratum corneum that can be assigned to lipids on the basis of their downward shift in cooling, obliteration on

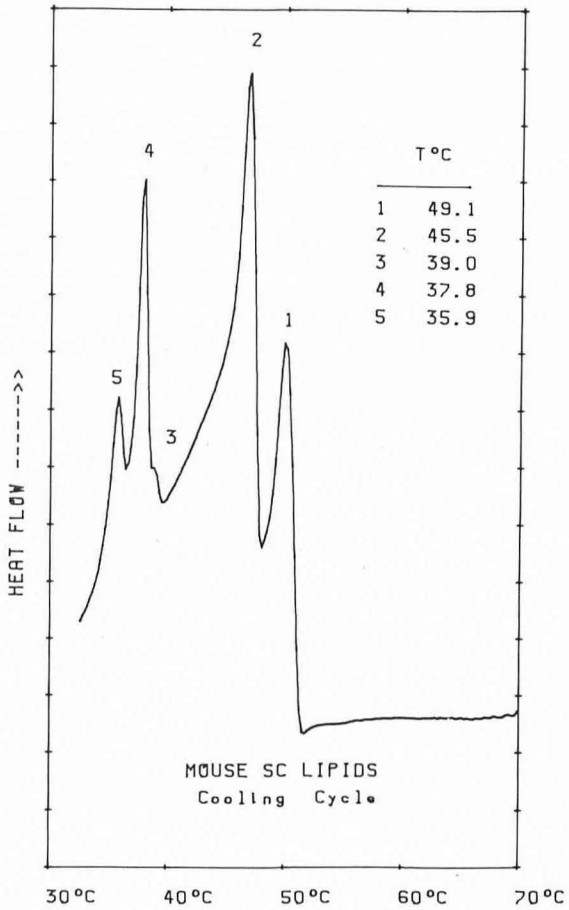

FIG 3. DSC cooling scan of the lipid extract of mouse neonatal stratum corneum. The second cooling scan (not illustrated) was identical to the first.

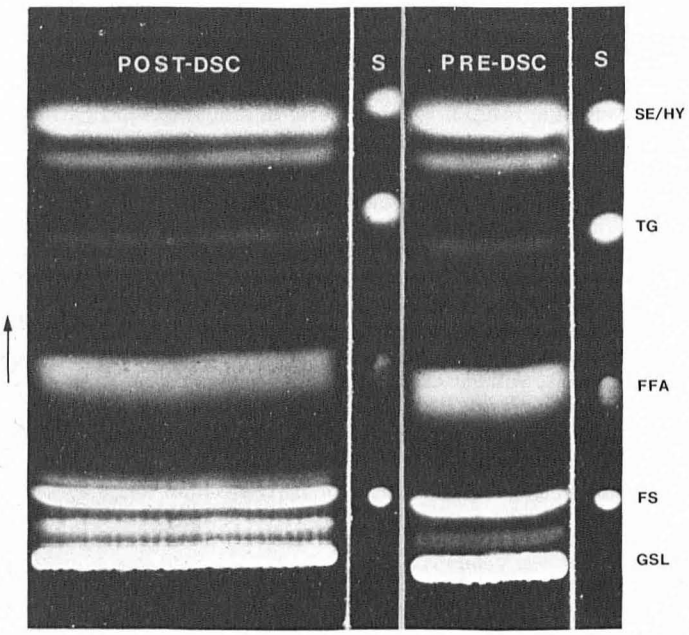

FIG 4. Thin-layer chromatograms of neutral lipids before and after treatment under conditions comparable to calorimetry (see Methods). Note essential similarity in number of neutral lipid species in both samples.

solvent treatment, and similarities to peaks observed in lipid solvent extracts of the same tissue. In preliminary studies similar peaks were also identified in stratum corneum sheets obtained from fresh human surgical and autopsy skin, and in lipid extracts from these human tissues as well [10], an indication that physiologic lipid transitions may be a general feature of mammalian stratum corneum. Although prior studies have sought lipid thermal transitions in the full temperature range [3–5], none have clearly documented their existence. Experiments are currently underway to determine whether discrete peaks on calorimetry can be assigned to specific lipid fractions. If such a relationship could be established, then, it might be possible to ascribe specific functions [11], e.g., water barrier, cohesion, desquamation, to phase-transition shifts of specific lipid fractions.

It is tempting to speculate that the appearance of several peaks on repeated heating and cooling results from zone fractionation of the initial lipid extracts in the sample chamber. Lipid fractionation in the DSC may occur as a result of either the large surface area of the sample cell in comparison to the sample size or the slow scan rate (~0.1°C/min.). The alternate explanation that these additional peaks represent degradation products from prolonged heating seems unlikely in light of the chromatographic profiles of the lipids pre- and postcalorimetry (Fig 4). The experiments in progress on specific lipid fractions will further clarify whether these fractions each derive from one individual lipid species.

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Carbohydrate Chains Specific for Blood Group Antigens in Differentiation of Human Oral Epithelium

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The distribution of A, B, and H blood group antigens and 2 blood group precursor carbohydrate chains (N-acetyl-lactosamine and lacto-N-triosyl-group) was examined in human buccal epithelium. The material included tissue from persons with blood group A, B, and O, 9 from each group.

Six patients in each group were secretors of blood group antigens in the saliva and 3 were nonsecretors. The blood group antigens and the precursors of these were studied by an immunofluorescence staining technique. Murine monoclonal antibodies were used to identify H-antigen (type 2 chain) and for N-acetyl-lactosamine whereas polyclonal rabbit antibodies were used for lacto-N-triosyl-group.

In all groups lacto-N-triosyl and N-acetyl-lactosamine were found on the cell membranes of basal and deeper spinous cells. The H-antigen was mainly present on cell membranes of the lower spinous cells. Only the basal cells above the connective tissue papilli showed cell membrane staining. Cytoplasmic staining in the upper two thirds of the epithelium was found with all antibodies when they were used in high concentrations.

The A and B blood group antigens were detected by human blood group test sera and were found exclusively on the cell membranes of the spinous cells. The different sites of expression of A and B antigens and expression of H, and the precursor antigens N-acetyl-lactosamine and lacto-N-triosyl, suggest a stepwise building up of carbohydrates with blood group specificities during the differentiation of buccal epithelial cells.

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